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L1: Entry 7 of 37 File: USPT May 22, 2001

DOCUMENT-IDENTIFIER: US 6235479 B1
TITLE: Methods and devices for performing analysis of a nucleic acid sample

Brief Summary Paragraph Right (4):
Nucleic acid examination is a continually emerging area for test sample analysis including investigating genetic sequence and expression. Initially, a test sample from a human patient or other source is isolated and target nucleic acids in the sample are amplified to increase the number of copies to allow the test sample to be analyzed. The copies of target sequences are then hybridized to one or more complementary oligonucleotide probes in solution or in combination with a solid support incorporating complementary probes to form a hybridization complex. A detector probe may also be hybridized to the target sequences under some reactions. Detection and identification of the hybridization product is achieved when a signal is generated from the hybridization complex formed. By amplifying nucleic acid sequences, information about the presence or absence of microorganisms is obtained from the sample without resorting to culturing microorganisms.

Detailed Description Paragraph Right (40):
In the third stage the target sequences are amplified. The technology is provided by the PCR techniques. This amplification feature allows the subsequent identification of DNA amplified from one or more genetic sequences per biological. Polynucleotides, custom-designed primers and polymerase enzymes for DNA replication are added in the first solution to enter the dehydrated matrices. The matrices act like sponges to take up the solution. A polymerase chain reaction cycle consists of a) binding the primers, b) extending the DNA chain and c) denaturing the DNA molecules. Each step in the cycle requires a temperature change. Thus, typical temperatures at the three stages would be chosen from the following three ranges respectively, 37.degree.-40.degree., 63.degree.-72.degree. and 94.degree.-96.degree. C., but other temperatures may be useful for alternate procedures. The cycles repeat until sufficient target sequences are present for detection. The number of cycles necessary depends upon the efficiency of the polymerase and the detection sensitivity of the label being used.

Detailed Description Paragraph Right (47):
In the following examples, amplification is defined as a means to biochemically increase the target nucleic acid mass. Target nucleic acid means those molecules containing a designated genetic sequence. Separation of nucleic acids by size utilizing electrophoresis is performed in a hydrogel supplied with an electrical current. Hybridization refers to the binding of complementary nucleic acids sequences, one partner of which carries a label whose signal can be detected. If amplification is used alone or follows hybridization it is understood that the primers or sequences used in binding targets or nucleotides for amplification may also carry a label.

Detailed Description Paragraph Right (48):
It is further understood that automated processing begins with sample preparation and ends with the test results of detection. It is further understood that standard reagents and reaction conditions may be used for the various sample treatment steps, such as amplification, electrophoresis and hybridization. The following examples are presented to iterate the ways in which the methods that are diagnostic of nucleic acid sequence-specificity may be interchanged or combined in the processing. In the following examples, the specimens in sections 46 are combined with matrix material which might be agarose; sections 54 are pre-formed in the carrier and may be different compositions to amplify different targets. Polymerase chain reaction (PCR) is shown in the preferred method of amplification when amplification is used, but amplification methods are not limited to PCR. Our research demonstrated amplification in agarose gels by PCR with Taq polymerase. The addition of more primer molecules during PCR as they are used retards formation of undesirable primer dimers. Although not discussed in detail herein, standard techniques including immuno-staining for analysis of polypeptides or other cellular components in gels may be performed with the device of the invention.

Detailed Description Paragraph Right (71):
Additionally, the more primer sequences used in PCR to amplify more fragments, the more specific is identification of target DNA. There is a dampening effect that limits the number of primer pairs that can be used together, which varies according to the nature of the target and background DNA or RNA. A particular assay may be used in which multiple primer pairs are used to increase the total quantity of DNA in the sample. Duplicate samples are run in parallel on the same matrix as positive and negative controls. The positive controls have primers to amplify a conserved region of specimen DNA that is species-specific and indicates the starting amount of total DNA present in the specimen. Another positive control of known target DNA demonstrates adequate assay conditions. A negative control starts with non-target DNA to indicate possible contamination of assays components.

Other Reference Publication (3):
Nucleic Acids Research, Sommer and Tautz, "Minimal homology requirements for PCR", vol. 17, No. 16, 1989, p. 6749.

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